ASSESSING THE TOXICITY AND BIOAVAILABILITY OF 2,4-DINITROANISOLE IN ACUTE AND SUB-CHRONIC EXPOSURES USING THE EARTHWORM, *EISENIA FETIDA*

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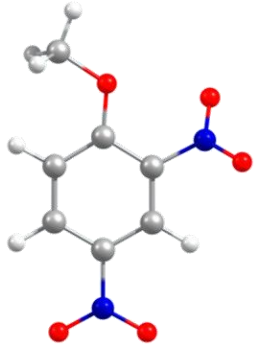

2,4 DNAN

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JUN 2010		**00-00-2010 to 00-00-2010**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Assessing the Toxicity and Bioavailability of 2,4-Dinitroanisole in Acute and Sub-Chronic Exposures Using the Earthworm, Eisenia Fetida	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Army Engineer Research and Development Center,Environmental Laboratory,3909 Halls Ferry Road Waterways Experiment Station,Vicksburg,MS,39180-6199**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
Presented at the NDIA Environment, Energy Security & Sustainability (E2S2) Symposium & Exhibition held 14-17 June 2010 in Denver, CO.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**24**	

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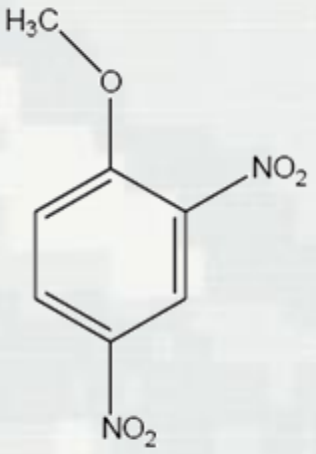
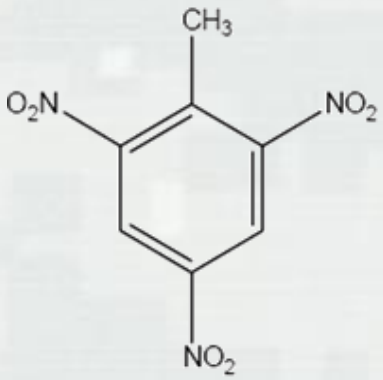
This work was supported by the Army's Environmental Quality Technology Basic Research Program

Overview



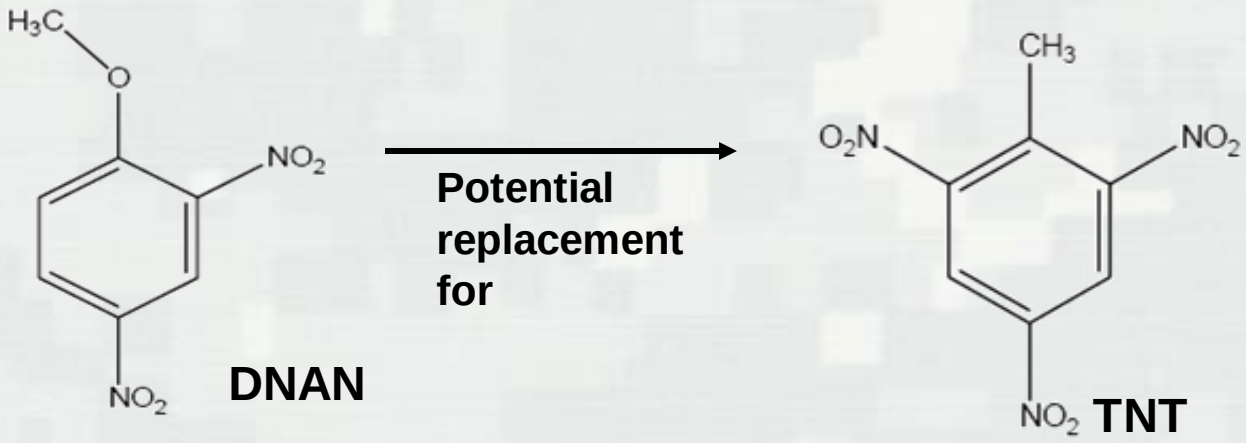
DNAN



TNT

- The Army currently produces and employs 2,4,6-trinitrotoluene (TNT) in traditional munitions, but is facing a mandate to replace TNT with less sensitive and less toxic compounds
- 2,4-dinitroanisole (DNAN) is currently being tested by the DOD
- Hypothesis: DNAN will exhibit a similar mechanism of action, but reduced toxicity relative to the closely related compounds, DNT and DNP
- Preliminary results obtained through acute and sub-chronic terrestrial studies suggest that DNAN is **less toxic** relative to TNT

2,4-Dinitroanisole



- Not a new entergetic → use in Amatol 40 (50% DNAN, 35% ammonium nitrate, 15% RDX) in the warhead of some V-1 flying bombs during World War II
- Gained popularity because of scarcity of TNT
- 10% reduction in explosive impact
- Little information exists on the environmental risk of DNAN, driving a need for further research in this area

Assessing the Environmental Toxicity of DNAN

Dermal Exposure

Ingestion

Whole Organism

Tissue

Cell

- Terrestrial ecosystems likely impacted due to munitions deployment over land
- Chose the earthworm *Eisenia fetida* as the terrestrial receptor
- Investigated multiple acute and sub-chronic exposure pathways:
  - Acute 72 hr dermal toxicity studies
  - Acute 7 day range finder soil studies
  - Sub-chronic 28 day soil studies
- Cellular toxicity endpoints coupled with exposures through *in vitro* Neutral Red Based Toxicology Assay (NRRT)

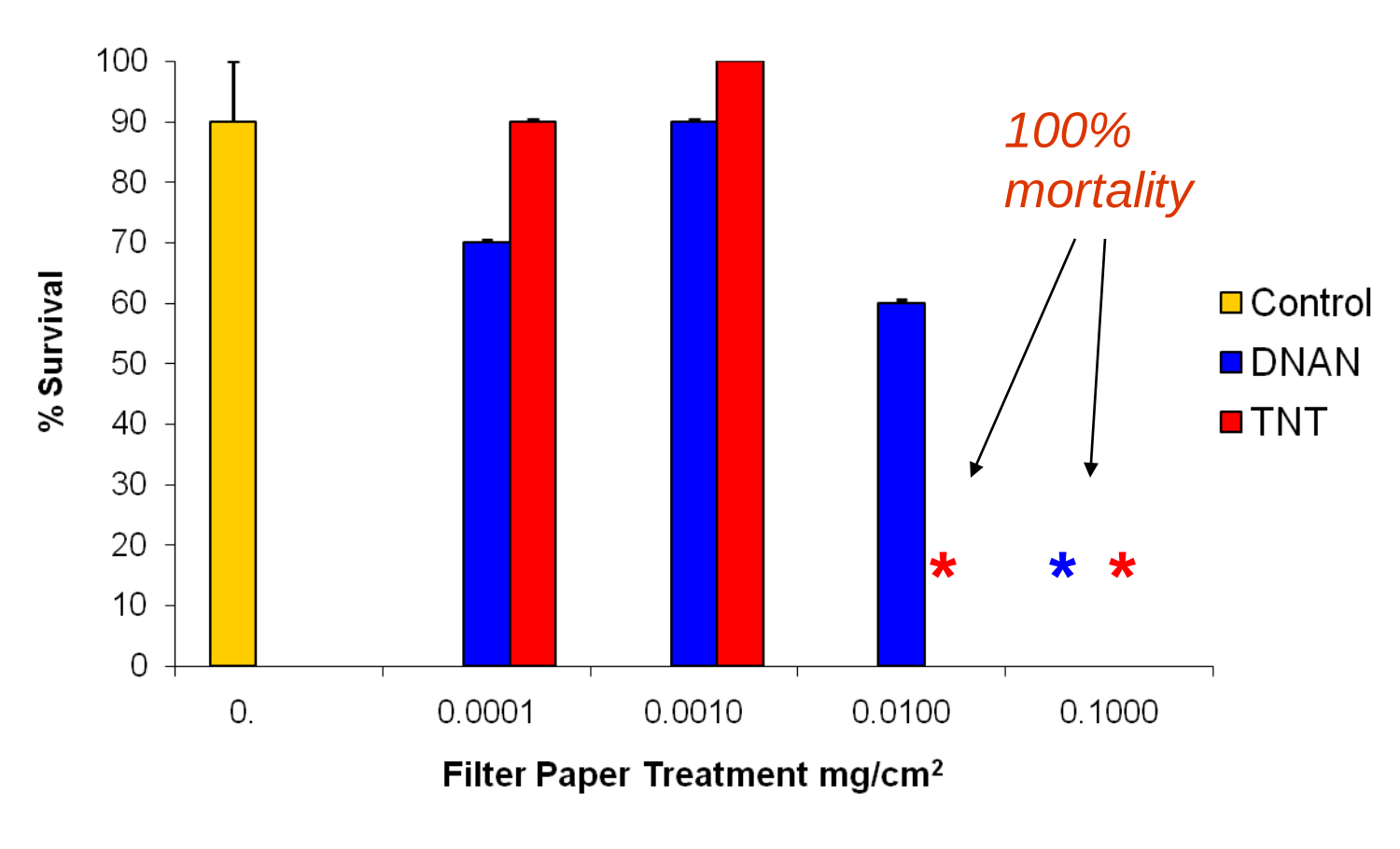
Dermal Filter Paper Exposure

- OECD Guideline 207 (1984)
- Duration: 72 Hours, n=10
- Treatments: DNAN and TNT dissolved in 100% methanol,1ml spiked onto filter paper: 0, 0.0001,0.001, 0.01, 0.1 mg/cm^2
- 1 earthworm per vial
- NRRT Analysis conducted from n=5 per treatment

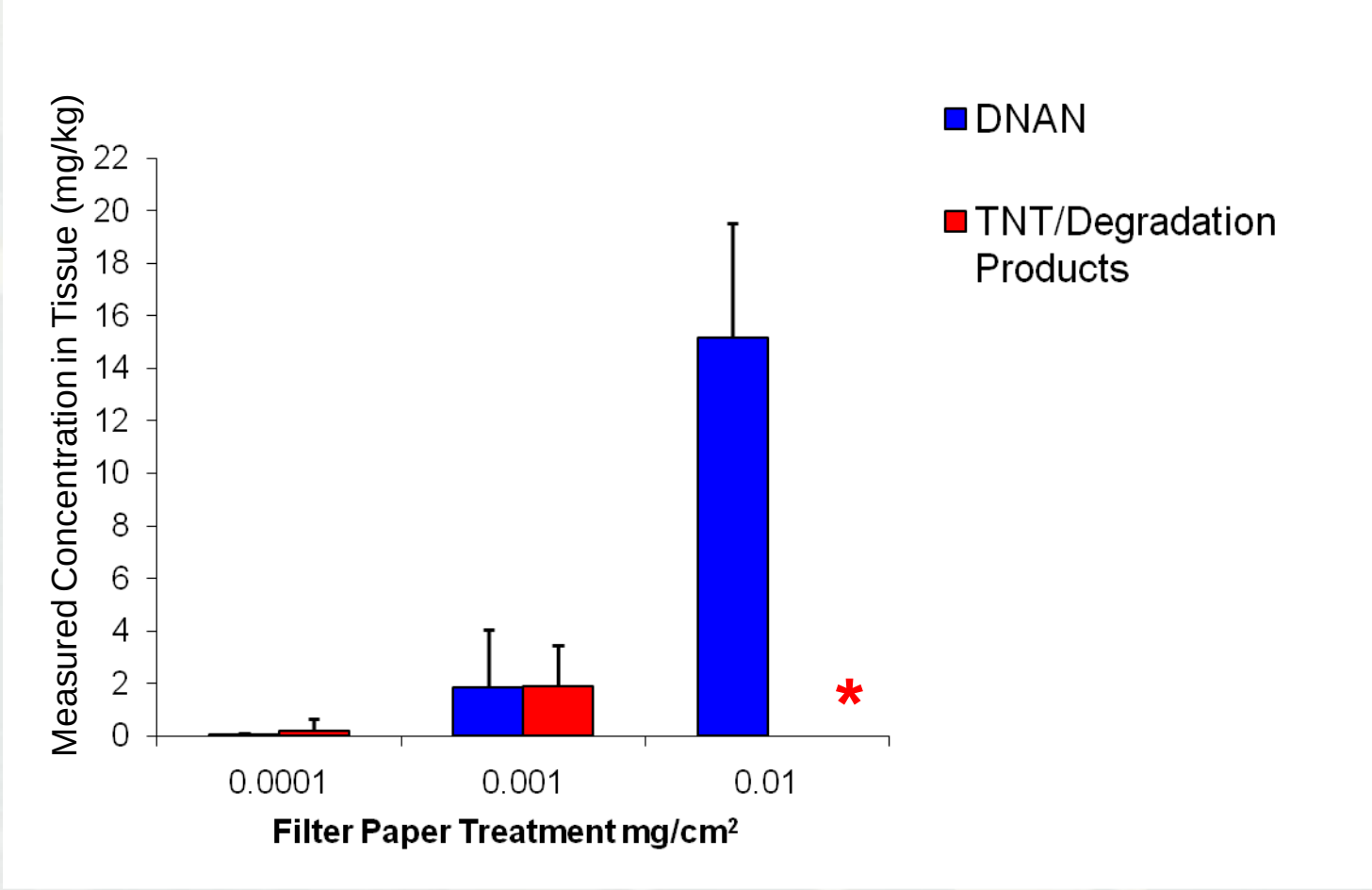
Toxicity/ Bioaccumulation

Cellular Stress NRRT

Dermal Toxicity of DNAN/TNT



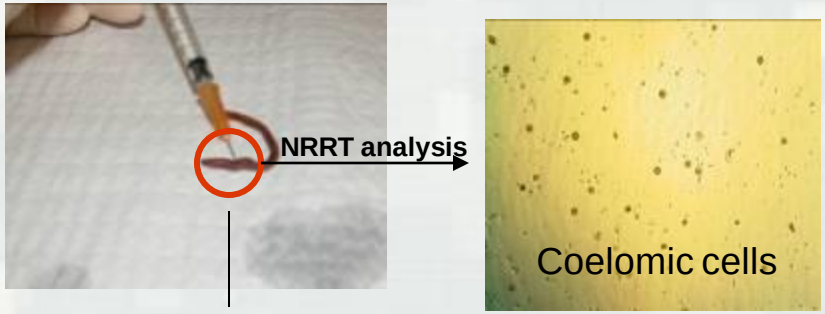
DNAN/TNT Bioaccumulation



Cellular Stress/NRRT

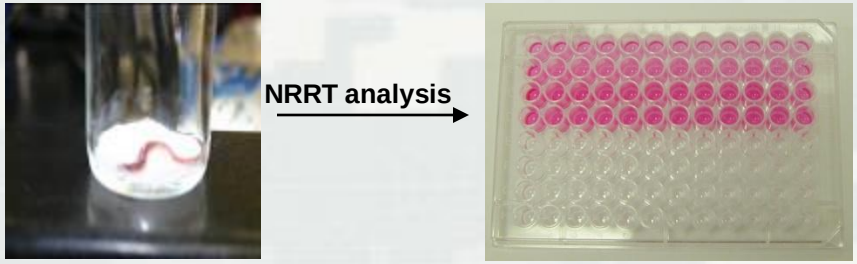
Two Methods of Measurement

1)



Extract 50 ul from clitellum

2)

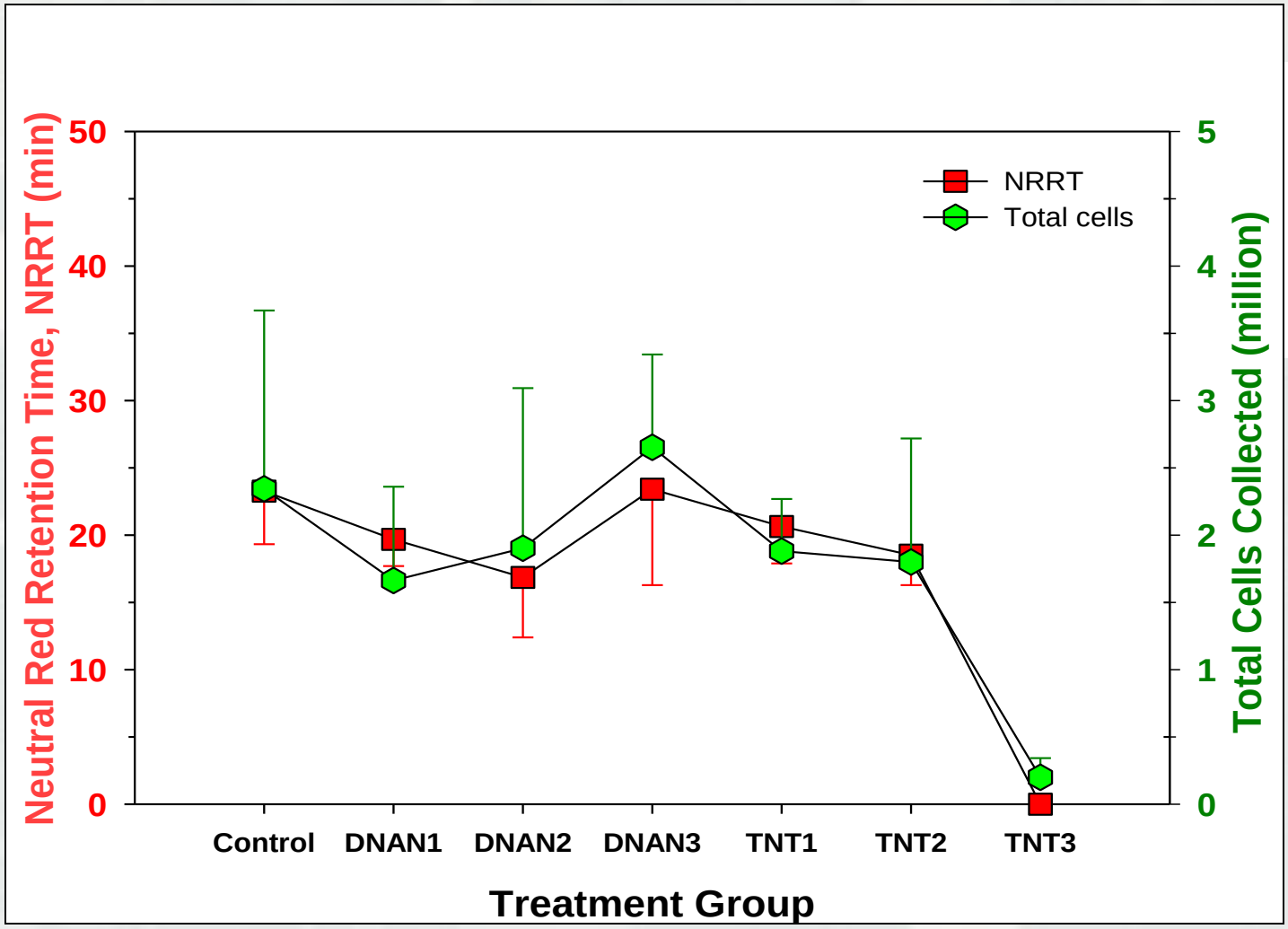


Expose worm to GGE t= 2mins collect coelomocyte solution

1 row per worm/treatment, obtain measurements through spectrophotometer

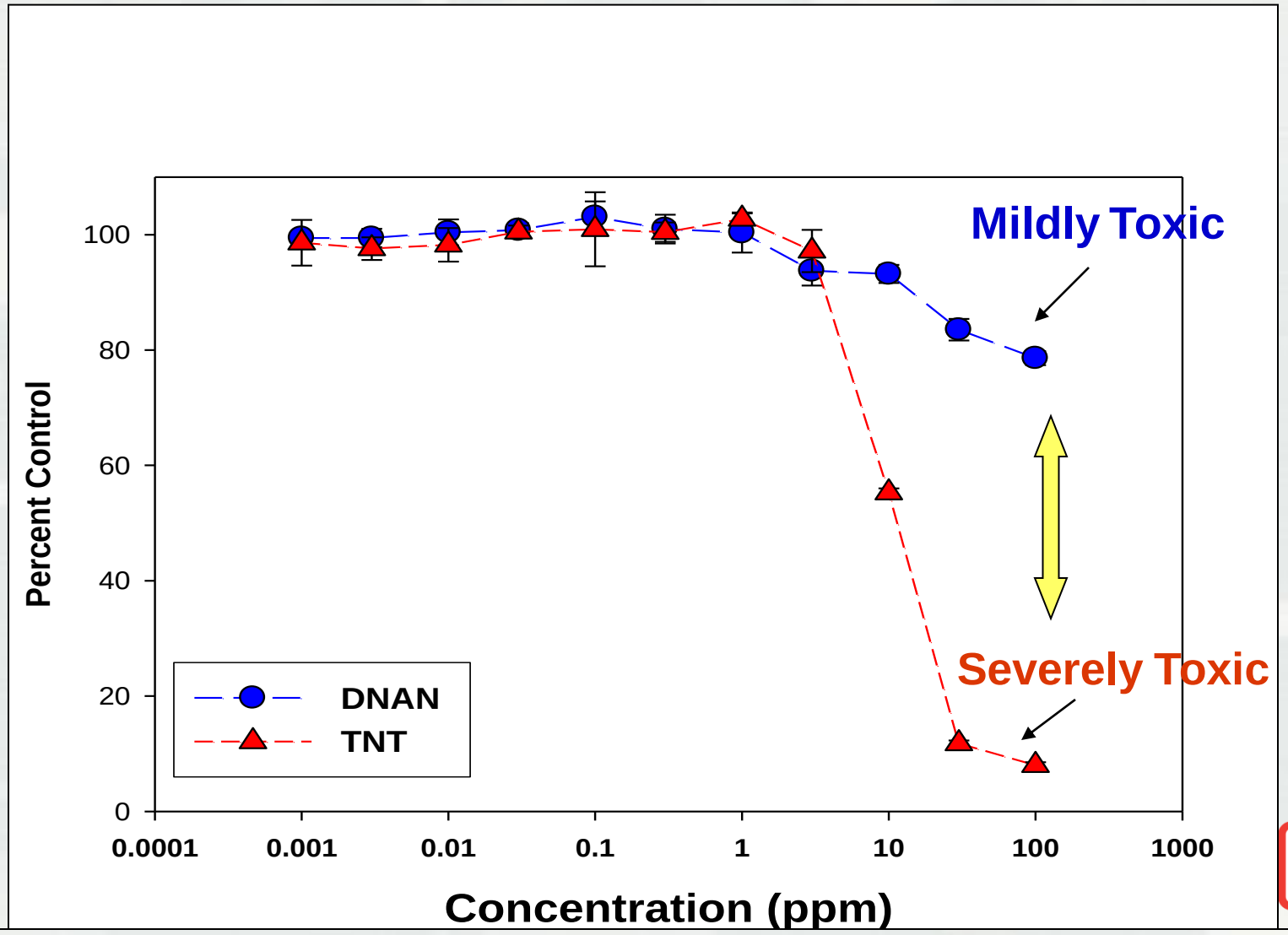
- NRRT is a biomarker of cellular stress; viable cells stain red with dye
- Coelomic fluid extracted and analyzed in two different methods, 2nd method chosen for final study:
  - Coelomocytes collected in 2 ml Guaiacol Glyceryl Ether (GGE) solution, centrifuged, decanted, washed with 1X Phosphate Buffered Saline (PBS) and re-suspended in 1,500 ul PBS
  - Kinetic readings obtained from spectrophotometer every 5 minutes for 1 hour to determine neutral red retention time

Comparison of Total Cells and NRRT



Treatment Group
mg/cm²
1=0.0001
2=0.001
3=0.01

DNAN & TNT Toxicity in Human Liver HEPG2 Cells



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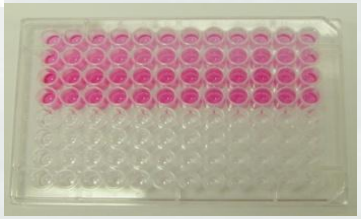
Soil Exposures

Toxicity &Body Burdens

NRRT

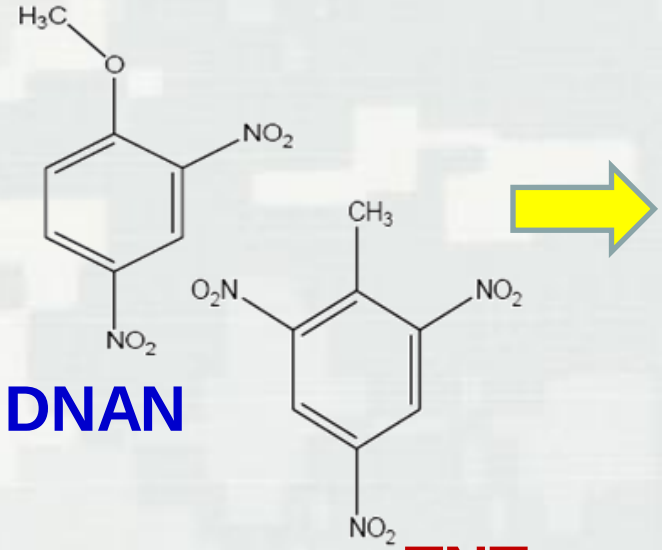


Reproductive Toxicity

- <u>Range Finder</u>-(n=3, 10 worms per rep) determine lethal and non-lethal range of DNAN
- <u>Sub-chronic 28 day studies -</u> (n=3, 10 worms per rep) comparatively assessed DNAN and TNT toxicity, bioaccumulation, and cellular stress through NRRT analysis
- Soil studies provide multiple routes of exposure through dermal contact and ingestion

Materials and Methods: Soil Prep

- Field soil spiked with increasing concentrations of DNAN and TNT dissolved in methanol
  - Range Finder Concentration: 0-300 mg/kg
  - 28 Day Exposure Concentrations: 0-100 mg/kg
- Tumbled overnight, placed in test containers, hydrated to 85% WHC



Materials and Methods: Test Conditions

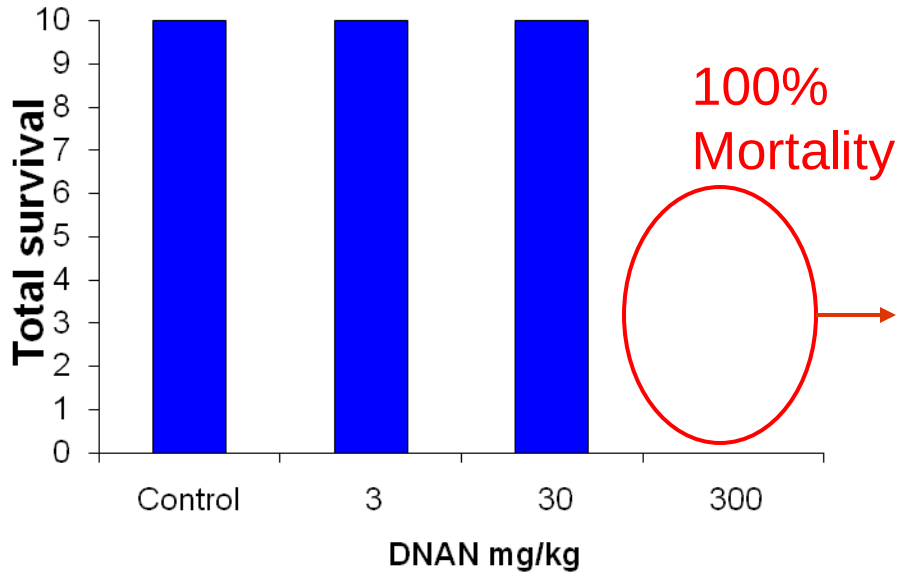
Earthworms depurated 24-hours
Adults 0.3-0.6 g

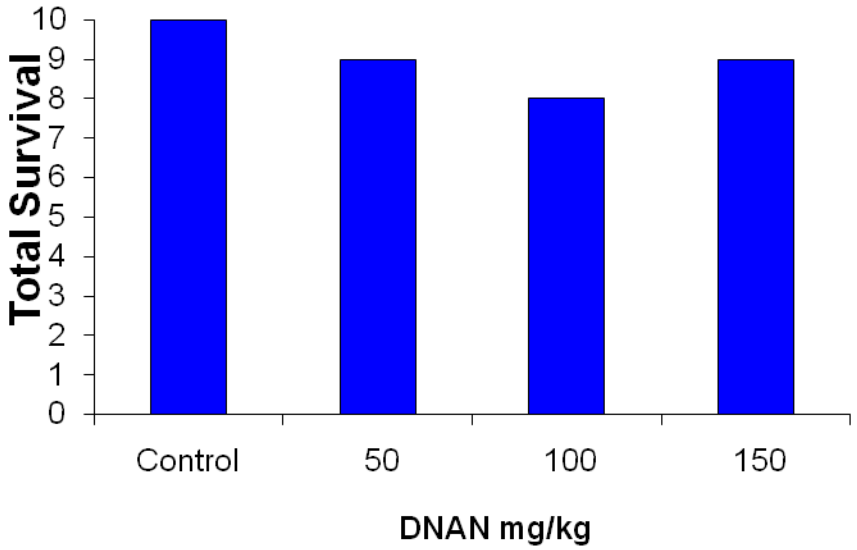
10 added per treatment n=3

Test conducted at 22°C, 80% humidity, continuous light

Endpoints : bioaccumulation, toxicity, growth, reproduction, NRRT

Results: DNAN Acute Range Finder





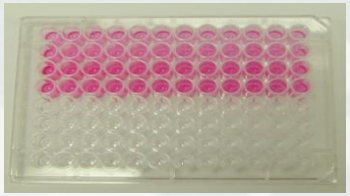
n=3, 10 per rep
T=7 days

Sub Chronic 28-Day Exposure

Toxicity &Body Burdens

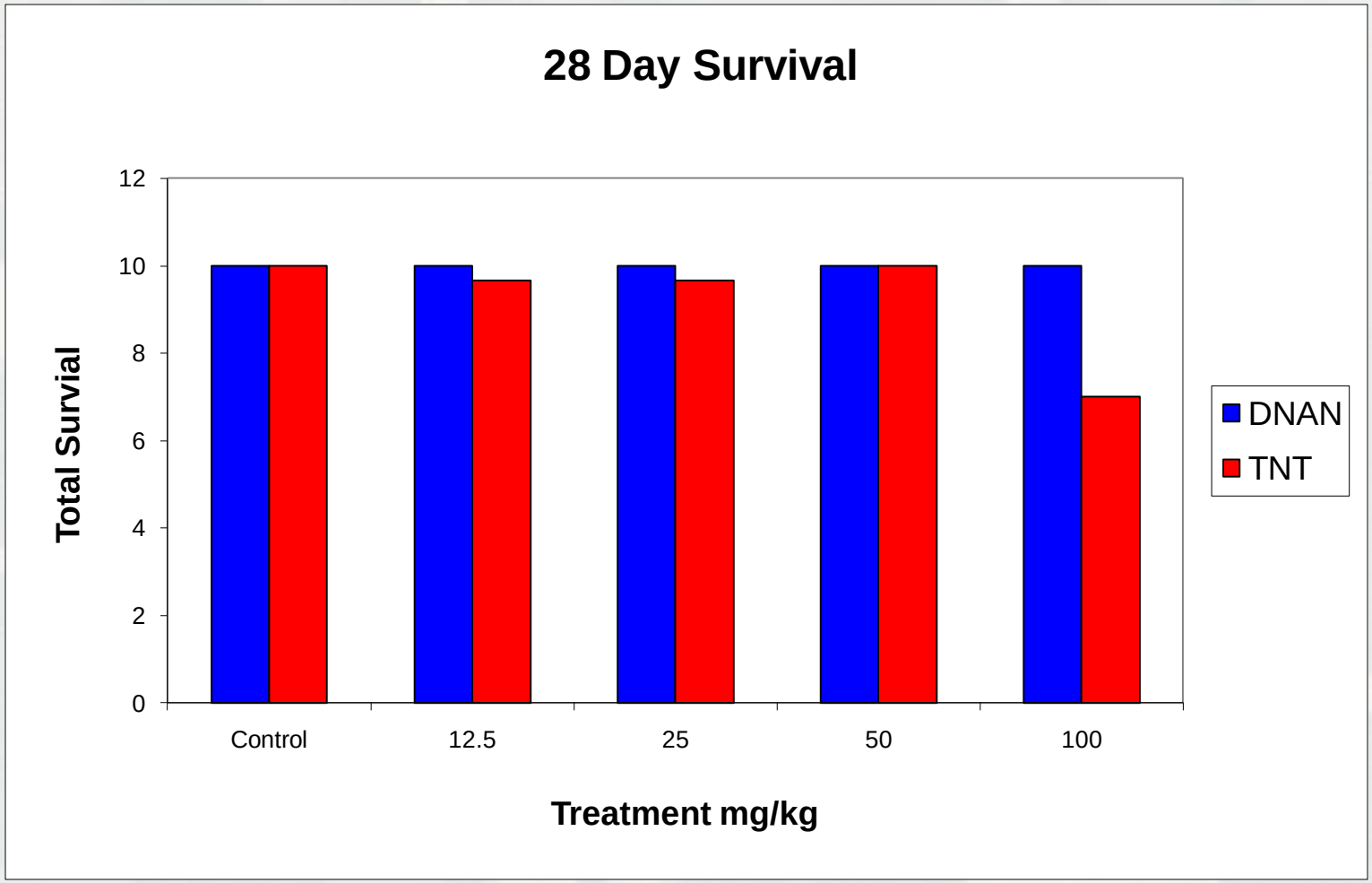
Reproductive Toxicity

NRRT



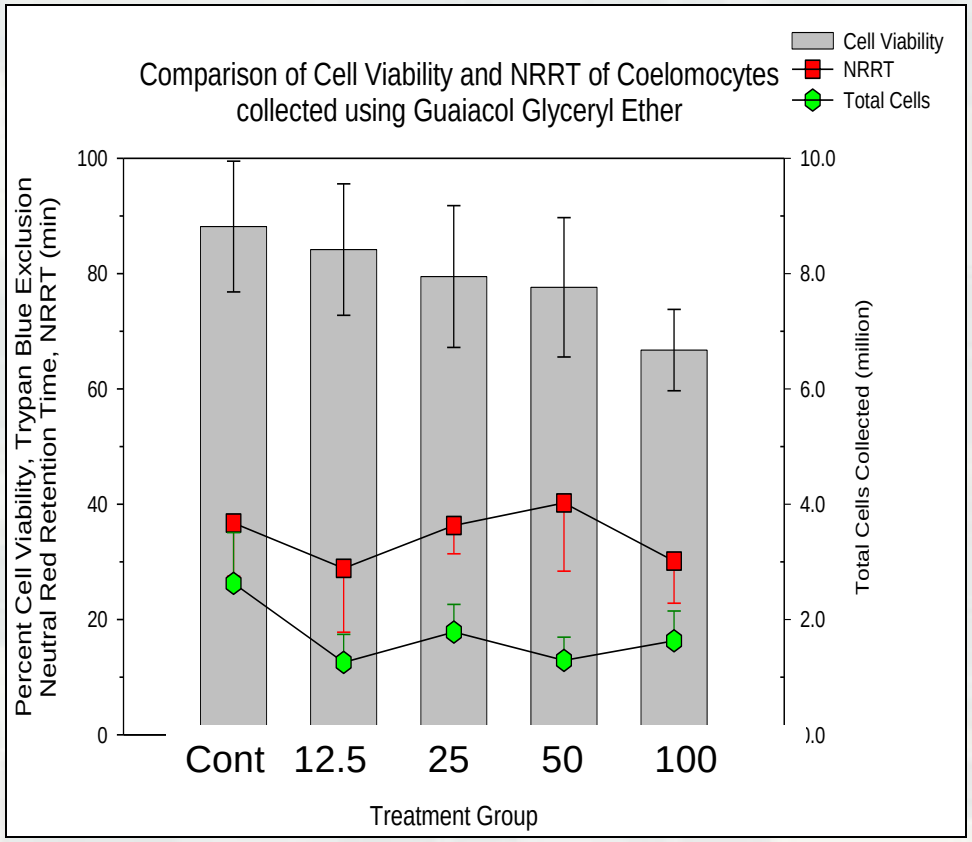
TNT and DNAN tested concentrations: 0, 12.5, 25, 50, 100 mg/kg n=3, 10 per rep

Sub-Chronic Preliminary Results

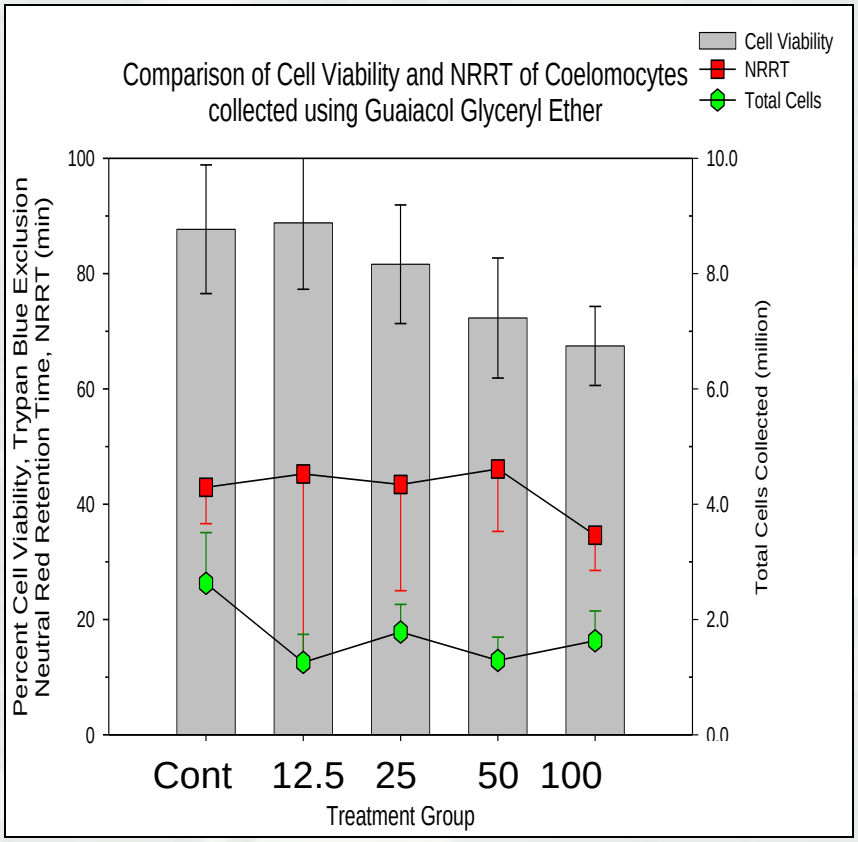


Results: 28 Day Exposure NRRT

DNAN

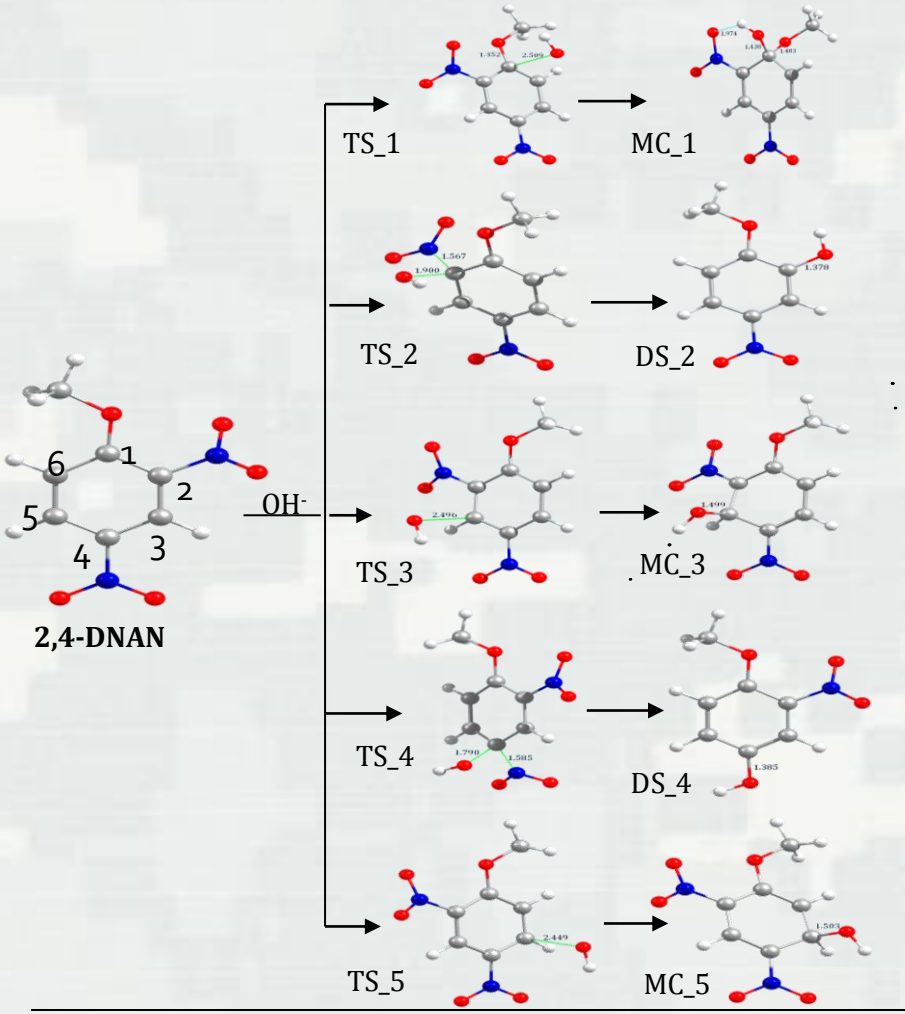


TNT



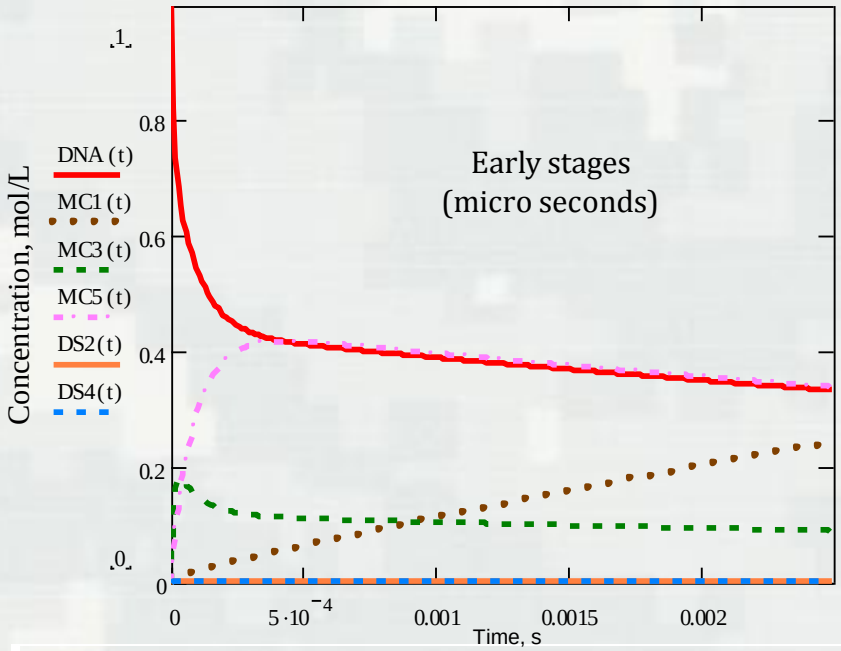
Computational Chemistry and Toxicity of DNAN

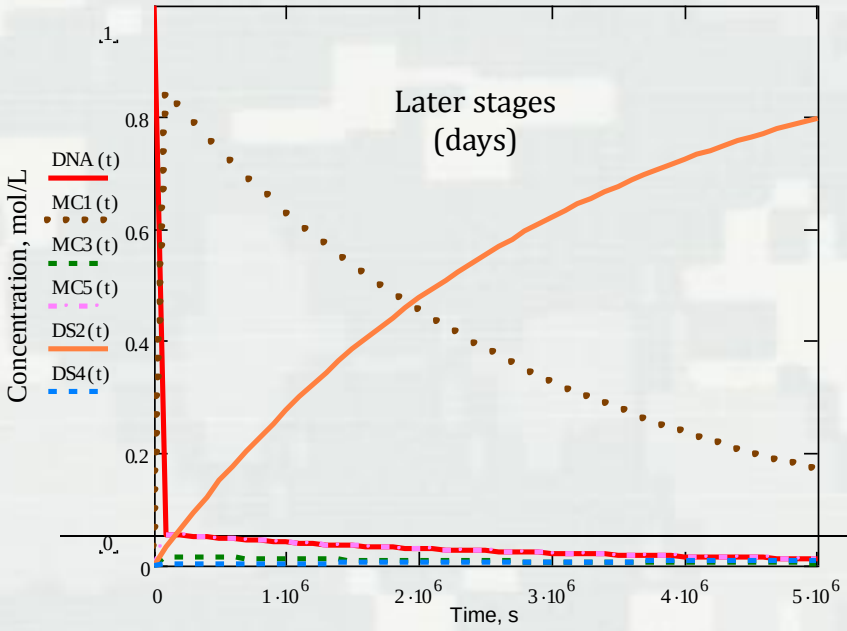
First stage of alkaline hydrolysis of 2,4-Dinitroanisole



- **Initial exposures conducted with DNAN parent compound**
- **Additional exposures to be conducted with DNAN degradation product predicted by computational chemistry models**
  - **TS (Transition State)**
  - **MC (anionic Meisenheimer complexes)**
  - **DS (direct substitution of nitro group w/phenol)**

Kinetics simulations





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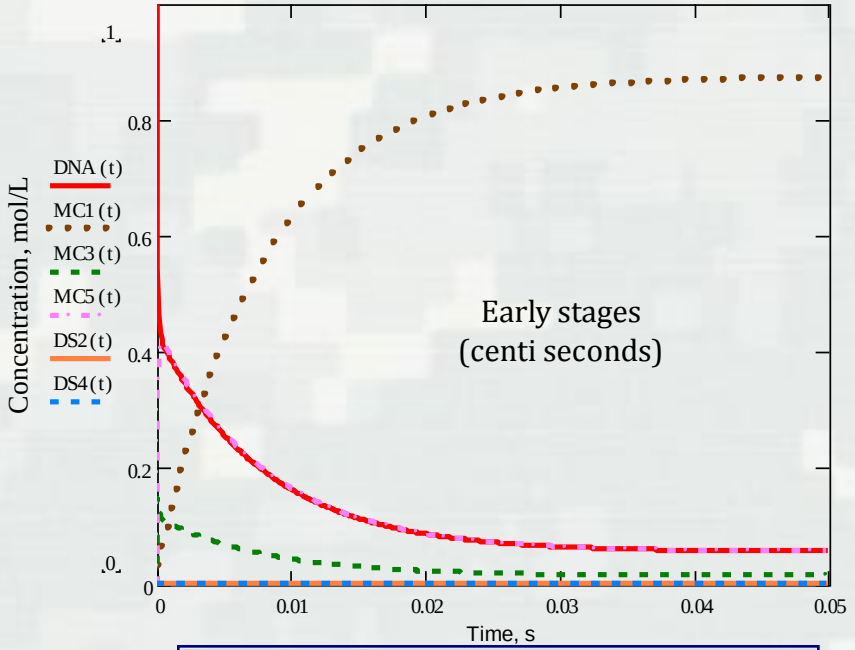
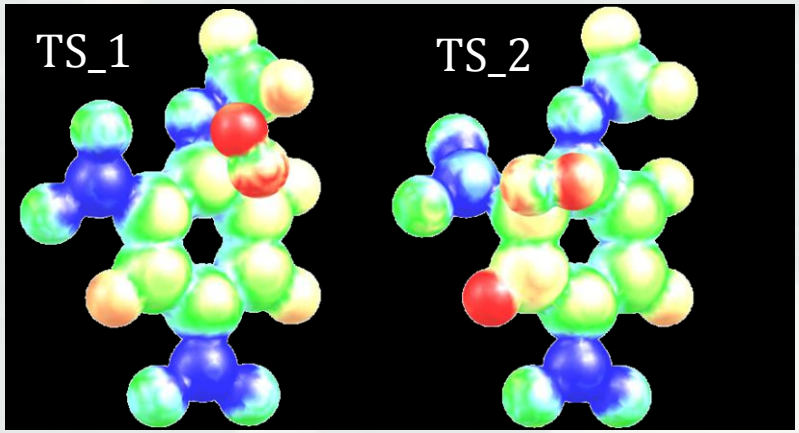
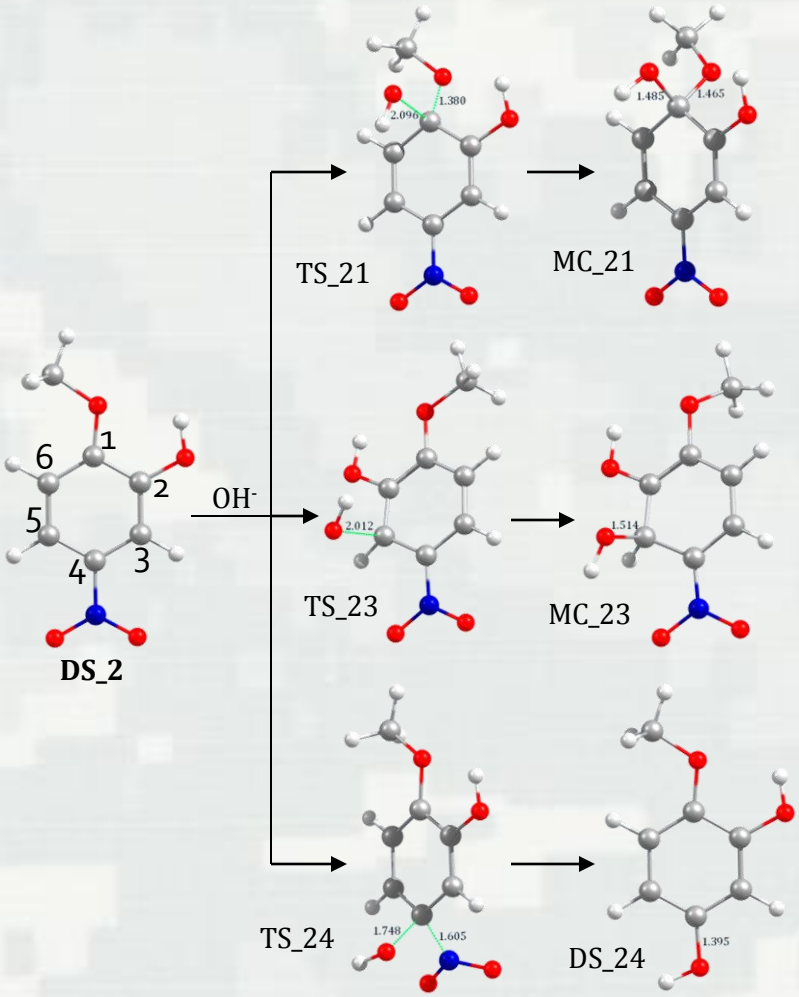


Electrostatic Potential (ESP) maps for selected transition states



Second stage of alkaline hydrolysis of 2,4-Dinitroanisole

We have considered attack of second hydroxyl anion on nitro phenol DS_2, formed on the first stage as a result of direct substitution.



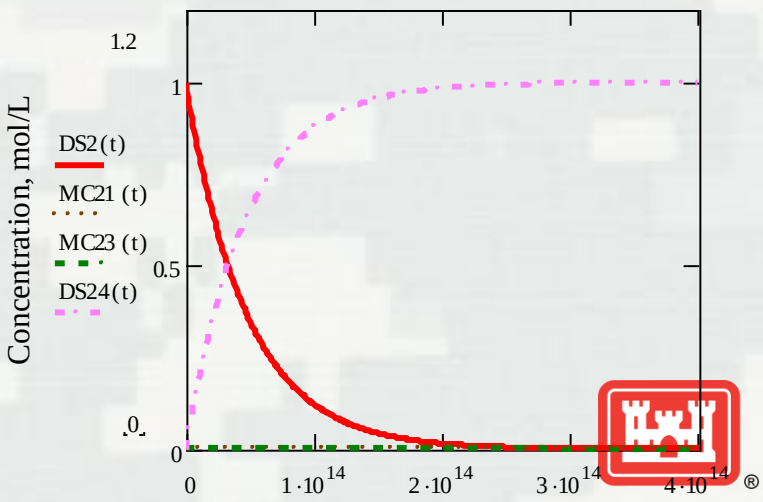
Transition states geometries				Products geometries			
TS	Bond C–O, Å	Bond C–R (R=H,CH_3), Å	∠OCR	TS	Bond C–O, Å	Bond C–R (R=H,CH_3), Å	∠OCR
TS_21	2.096	1.380	95.584	MC_21	1.485	1.465	103.631
TS_23	2.012	1.085	88.177	MC_23	1.514	1.099	105.804
TS_24	1.748	1.605	93.154	DS_24	1.395	-	-

** For bonds: first column is bond length in Å, second column is Wiberg index of bond order*

Reaction thermo chemistry

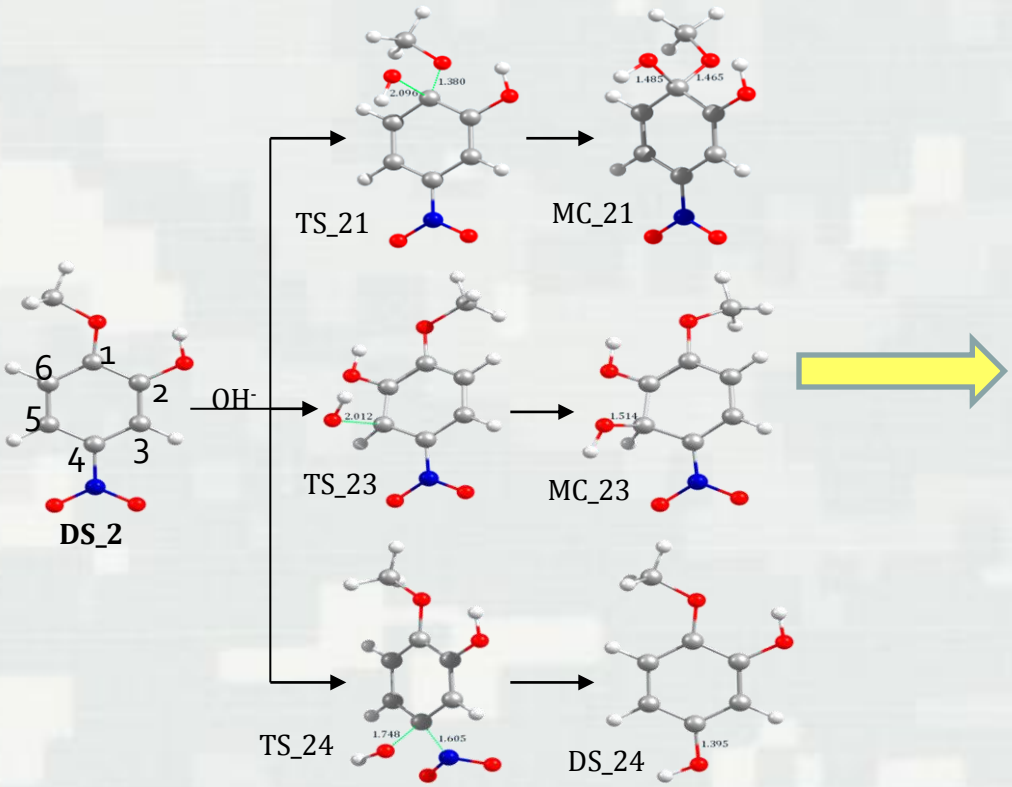
Activation parameters					Products parameters				
Reaction	ΔE, kcal/mol	ΔH, kcal/mol	ΔG, kcal/mol	ΔS, cal/mol*K	Product	ΔE, kcal/mol	ΔH, kcal/mol	ΔG, kcal/mol	ΔS, cal/mol*K
TS_21	13.74	13.53	**20.79**	-24.38	MC_21	6.08	5.84	**13.06**	-24.24
TS_23	15.65	15.49	**22.47**	-23.42	MC_23	11.46	11.18	**18.47**	-24.43
TS_24	29.48	29.37	**36.12**	-22.66	DS_24	-30.31	-30.78	**-35.25**	16.97

Kinetics simulations



2,4-dihydroxytoluene is highly dominating product because of low stability of competitive pathways adducts. However, its formation requires enormous amount of time.

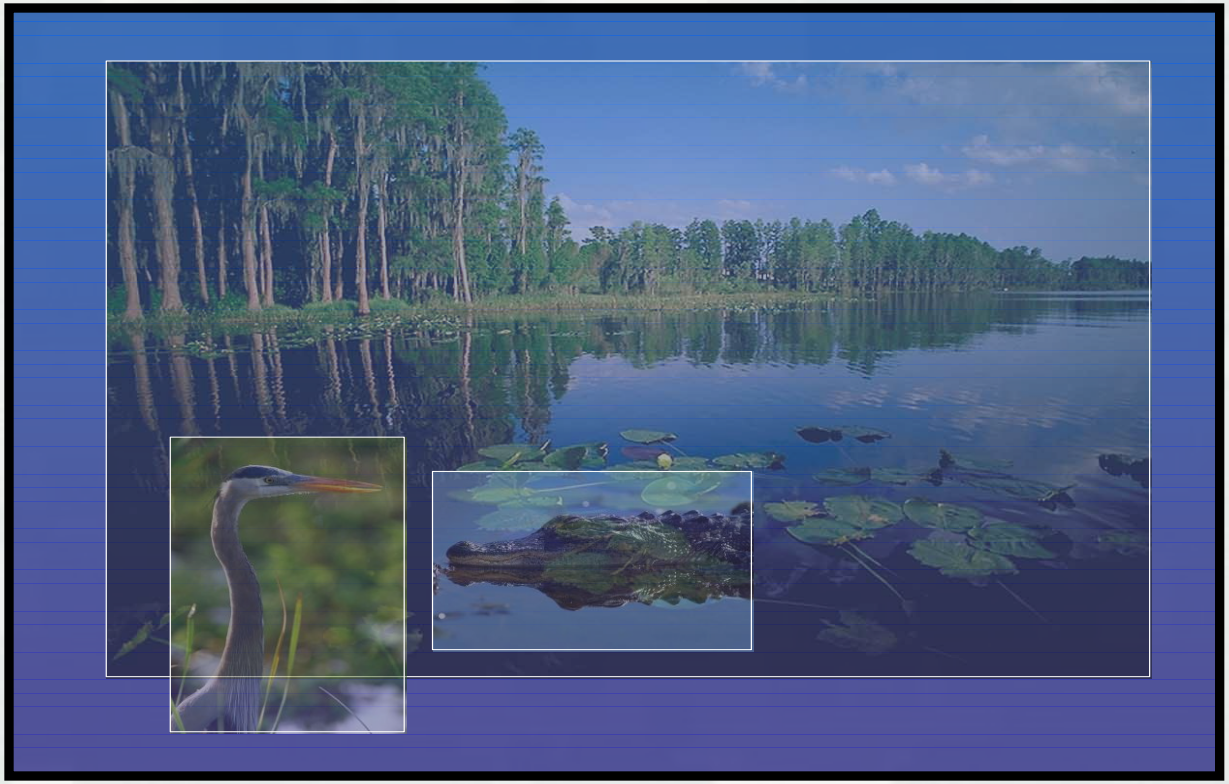
Future Direction of Project



Determine toxicity of predicted breakdown product in terrestrial systems

Overview: Results/Findings

- DNAN resulted in lower toxicity to relative to TNT compounds in *E. fetida* exposures
- Two methods tested for NRRT analysis; kinetic readings obtained from spectrophotometer found to be less variable
- Better definition of link between computational chemistry and toxicology as it relates to munitions
- Information previously unavailable on the bioavailability and toxicity of DNAN to terrestrial organisms
- Data obtained from our exposures will benefit the Army by reducing uncertainty about the environmental effects of DNAN; therefore, reducing cost associated with unnecessary site remediation.



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